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Original Article

Deeper and Early Molecular Response is a Good Predictor for Risk of Relapse in Patients with Acute Promyelocytic Leukemia

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Abstract.

Background: Conventional treatment of acute promyelocytic leukemia (APL) including all-trans retinoic acid (ATRA) with anthracycline had led to good patient response and survival. However, instances of relapse continue to occur, and remain the major problem with this course of treatment.

Patients and Methods: We collected the data of patients with newly diagnosed APL for the period 2004 to 2014, retrospectively. Data analysis included age, sex, initial white blood cell (WBC) count, coagulopathy and PML-RARα subtype. We also correlated PML-RARα fusion transcript level during treatment with outcome.

Results: A total of 33 patients were ultimately included in this study. Twenty-nine patients achieved complete hematologic remission(CHR) and 24 patients achieved complete molecular remission(CMR). The median overall survival duration was 92.8 months. Patient relapse and initially high WBC count were 2 factors associated with poor survival (P = 0.003; P = 0.015). Eight patients relapsed and the relapse-free survival (RFS) was 41.8 months. A reduction exceeding 4 log of PML-RARα transcript level after induction and undetectable PML-RARα transcript after treatment was associated with better RFS (P = 0.028).

Conclusions: Deeper molecular response after induction therapy was associated with a reduction in relapse and could be used to predict risk of relapse in newly diagnosed patient under conventional treatment.

Keywords **:** acute prolmyelocytic leukemia, PML-RARα, relapse

原著論文

治療初期較深的分子生物反應在急性前骨髓性白血病病人的治療上是好的預測因子

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中文摘要

背景：急性前骨髓性白血病(APL)在現今結合全反式 A 酸及 Anthracycline 類藥物的治療下，已有不錯的成績。但仍有部分病人產生復發進而導致死亡，本研究回顧近 10 年在本院治療的 APL 病人，分析診斷時的各項因子(年齡、性別、白血球數等)並追蹤治療過程中 PML-RARα 融合基因量的變化，分析其與預後之關係。

方法&結果：共 33 位病人於本院治療，其中 29 位達到血液完全緩解，24 位達完全分子生物學緩解。平均存活時間為 92.8 月。不良預後因子分別為診斷時高的白血球數(P=0.015)及復發(P=0.003)。8 位病人復發，無復發存活期為 41.8 個月。誘導治療後 PML-RARα 轉錄值下降大於 4.0log 或測不到為一好的預後因子(P=0.028)。

結論：復發在 APL 預後上至為關鍵，而誘導治療後較深的分子生物學反應意味著較少的復發，因此可視為一個好的預測因子。

***關鍵字*:** 急性前骨髓性白血病、PML-RARα、復發

INTRODUCTION

By using *PML-RARα* fusion transcription, APL could be a good model for residual disease (MRD) monitoring [1,2]. Post consolidation MRD monitoring had been regarded as an important component of international guidelines for the management of APL [3,4]. However, the clinical implication of early molecular response after induction still needs to be defined.

Therefore, we performed this retrospective review in our hospital. The aim was to define the prognosis of APL patient under conventional therapy and stratified risk for relapse using quantification of *PML-RAR*a transcript.

PATIENTS AND METHODS

Patients and Samples

From February 2004 to September 2014, we collected data from patients with newly diagnosed APL retrospectively in our center. The diagnosis of APL was made by morphology, immunophenotype, and cytogenetic determination. Some diagnoses were also confirmed by RT-PCR for *PML-RARα* rearrangements.

If a patient obtained complete hematologic remission after induction, we checked (RQ-PCR) for *PML-RARα* transcript levels from bone marrow samples at 3 different time points: after induction, during consolidation and after consolidation therapy.

Treatment Protocol

Our treatment protocol was based on the APL-99 protocol, which included an induction with ATRA plus idarubicin or daunorubicin and two to three consolidation courses with idarubicin, followed by a maintenance phase with ATRA, methotrexate and mercaptopurine for 2 years. ATRA would be added in those cases where the initial WBC count was higher than 10000/L during the consolidation phase.

RQ-PCR Assay

Quantification of the *PML-RARα* transcripts was performed according to the Europe Against Cancer program protocols [5,6]. The assays were run on an ABI PRISM 7700 or 7900 DNA sequence Detection System. Fusion transcripts were normalized with *ABL*17. All samples were tested in duplicates, and the results were reported according to the Europe Against Cancer guidelines as the normalized copy number, derived by multiplying the *PML-RARα* copy num-

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Table 1. Analysis of factors associated with overall survival

	Patient number	Median O.S.	P value
Initial WBC count (/L)			
<=10000	22	108 (85.6-118.4)	0.015
>10000	11	68.5 (29.2-101.7)	
Platelet			
<= 30000	16	80.9 (56.6-105.5)	0.204
>30000	17	103.6 (85.2-112.0)	
DIC			
Yes	18	102.1 (81.9-112.4)	0.544
No	15	91.5 (54.7-105.6)	
Age			
>60	5	74 (29.3-119.2)	0.204
<60	28	101 (72.8-112.9)	
Sex			
Male	18	91.2 (57.7-107.5)	0.418
Female	15	99.4 (81.4-117.3)	
PML isoform			
S (bcr3) form	9	53.7(42.9-64.6)	0.412
L (bcr1) form	15	83.6(70.5-96.6)	
V (bcr2) form	4	86.9(38.2-135.7)	
CHR			
Yes	29	106.8(87.2-116.2)	< 0.001
No	3	0.3 (0.1-0.8)	
Relapse			
Yes	9	36.7(23.7-72.8)	0.003
No	21	Not reach	
Maintenance therapy			
ATRA plus chemotherapy	21	107.1(93.1-121.1)	0.293
ATRA only	9	65.2(49.9-80.4)	

ber/*ABL* copy number ratio by 100.

Definition and Statistical Analysis

Hematologic remission was defined as normal bone marrow cellularity with <5% leukemic promyelocytes and normalization of peripheral blood counts. Consequently, relapse was defined as the reappearance of 5% leukemic promyelocytes in the bone marrow. Relapse-free survival (RFS) was calculated from the time CR was achieved to the patient's last follow-up or an event defined as hematologic relapse.

Molecular remission (MR) was defined using quantitative RT-PCR (RQ-PCR) to detect *PML/RARα* transcript levels. Early molecular response is defined as a more than 4 log reduction after induction therapy, and complete molecular remission (CMR) as more than 5 log reduction, or undetectable.

Relapse-free survival (RFS) for analysis after

Table 2. Analysis of factors associated with relapse free survival

	Patient number	Relapse-free survival	P value
Initial WBC count			
>10000	8	30.9 (20.5-41.4)	0.964
<=10000	21	40.4 (32.3-48.6)	
DIC			
Yes	15	30.1 (23.3-36.9)	0.405
No	14	44.0 (34.2-53.9)	
Maintenance therapy			
Yes	21	40.8(32.2-49.5)	0.886
No	9	30.3(21.5-39.2)	
Molecular response checked during 3 treatment phases			
Post induction			
>4 log	8	Not reach	0.024
<=4 log	16	31.4(23.8-38.9)	
During consolidation			
CMR	9	42.8(33.3-52.3)	0.521
Non CMR	15	27.2(20.1-38.2)	
Before maintenance			
No CMR	1	10	
CMR	23	42.0 (34.7-50.3)	

consolidation therapy was defined as the time between the achievement of complete remission and hematology relapse or the last follow-up. OS was defined as the time from achievement of complete remission to death or last follow-up. The probabilities of RFS and overall survival (OS) were calculated using the Kaplan-Meier method and compared using the log-rank test. All tests were carried out with the SPSS 14.0 program (SPSS, Chicago, IL, USA).

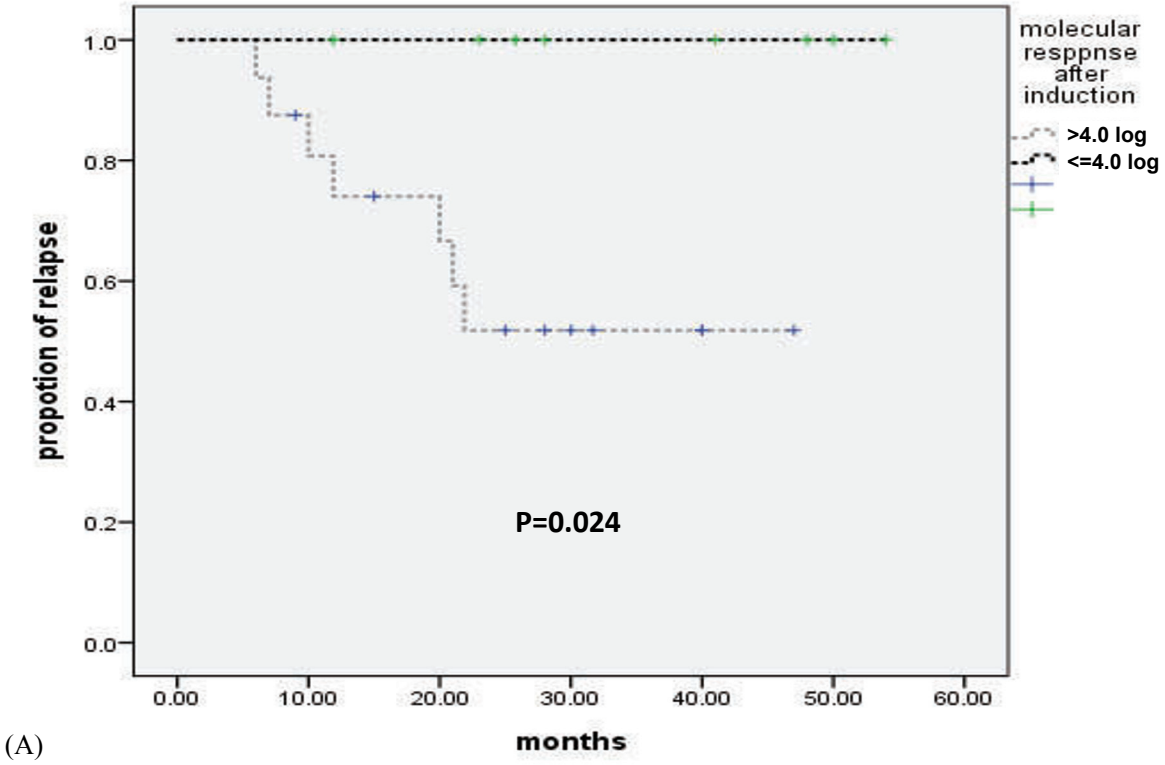
RESULTS

A total of 33 patients were included in this study with median follow-up time of 41 months, 18 were male and 15 were female with the median age of 44 (range 20 to 77). The medium WBC count was 2000/uL (range from 200/uL to 625000/uL) with a median platelet count of 28200/uL (range from 3000/uL to 146000/uL), and 18 patients presented with disseminated intravascular coagulation (DIC) during diagnosis.

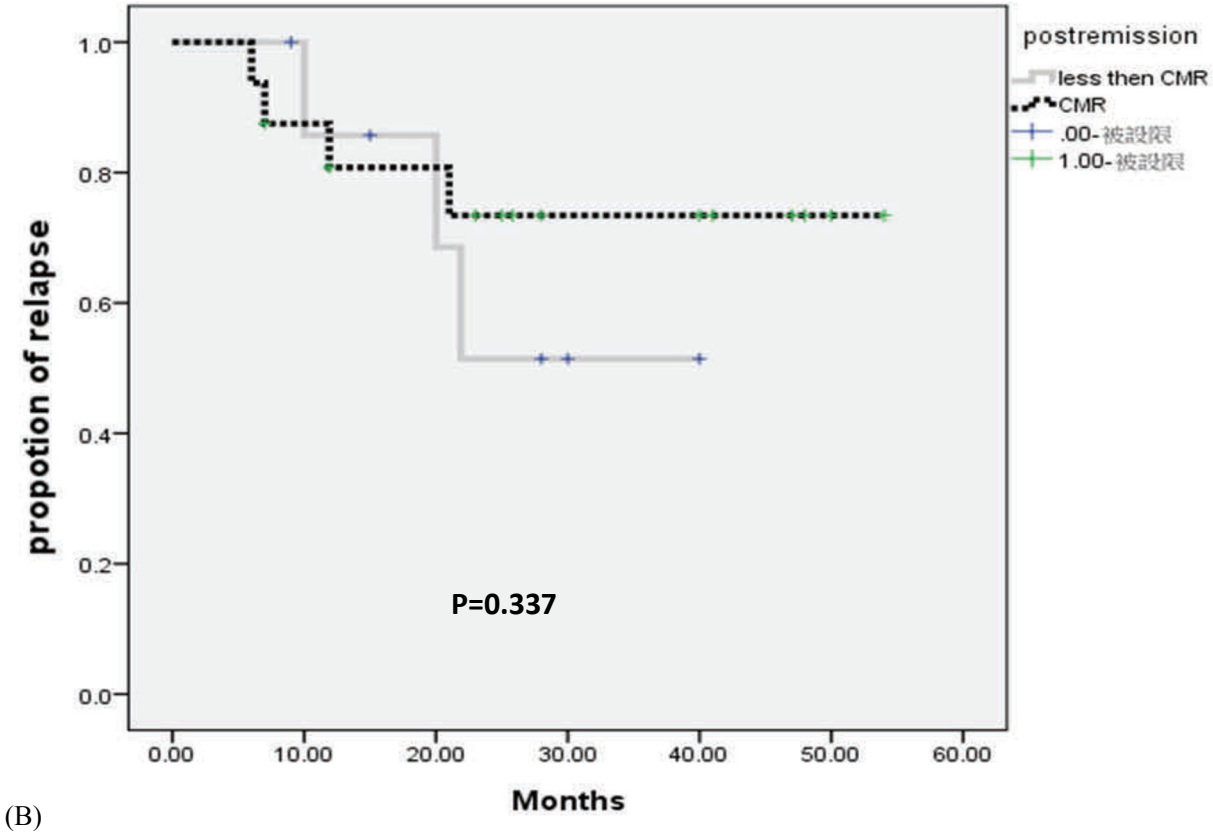
Treatment Outcome

After induction, 29 patients had achieved complete hematological remission (CR rate: 87.9%) and 4 patients died during the induction period, (1 died of DIC and cranial hemorrhage, 1 died of myocardial infarction and 2 died of infection).

The *PML/RARA* transcription level was monitored in 24 patients during treatment. After induction, 11 patients were early molecular responders (>4.0 log) and 2 of them got CMR. At the end of consolidation, 23 patients got CMR and the rest (1) also had good



(A)



(B)

Figure 1. Relapse-free survival (RFS) of patients with different molecular response checked at different time points during therapy (A) After induction (B)During consolidation

molecular response (more than 4.0 log reduction). All of the early responders got CMR after consolidation therapy was completed.

The medium overall survival duration was 92.8 months, with 5-year overall survival of 80%. The univariant analysis showed that high WBC count ($p = 0.015$) and relapse ($p = 0.007$) were significantly associated with poor outcome; contrarily, those with CHR after induction had better outcome ($p<0.005$) (Table 1).

Eight patients relapsed after CHR at a median of 14 months (range 5–21 months). The RFS was 41.8 months. Poor prognostic factors such as high initial WBC count and DIC are not related to RFS. Maintenance therapy didn't improved RFS, either (Table 2).

The early responders and those with CMR after consolidation ended were associated with better RFS (Figure 1A). But those patients who achieved CMR during consolidation showed no benefit in RFS (Figure 1B).

DISCUSSION

An Initially high WBC count upon diagnosis was regarded as a poor outcome for APL[7]. Besides high WBC count, we had also found that relapse was associated with worse OS and CHR and with better OS. Patients with early molecular response with more than 4 log reduction post induction chemotherapy were associated with better RFS.

The detection of *PML-RARα* by RQ-PCR becomes an important tool for monitoring MRD in patients with APL[3,8-10]. Early molecular response had also been proven as a predictor of better outcome in certain specific hematologic malignancy, such as newly diagnosed CML under TKI therapy[8].

In APL, however, the kinetics of tumor burden reduction (log-reduction between diagnosis and post-induction) did not correlate with disease outcome[9]. These results contrast with other leukemic disorders such as t(9;22) CML or t(8;21) AML[10-12]. Such differences could be explained in part by the type of therapy, since ATRA, unlike other cytotoxic treatments, promotes the differentiation of APL cells instead of quickly eliminating leukemic cells. Therefore, induction treatment of APL can be associated with delayed leukemic clearance [11].

However, two additional recent studies also found detection of *PML-RAR*a at the end of the initial induction therapy correlates with subsequent risk of relapse in patients, especially those receiving Arsenic trioxide (ATO) therapy[13,14].

Recently, a phase II study demonstrated the outstanding treatment featuring ATO and autologous hematopoietic stem cell transplantation for relapsed APL, where 5-year event-free and overall survival rates were 65% and 77%[15]. This same treatment also showed benefit in our patients. Among the 8 relapse patients, 7 had received salvage chemotherapy with arsenic trioxide and all of them achieved complete hematological remission. Three of them had further received autologous stem cell transplantation and got CMR. The other patient received reinduction with duonorubicin plus cytarabine but died of neutropenia.

The role of maintenance therapy in newly diagnosed APL had been an often-debated issue [3,16]. All of our patients received maintenance therapy after complete remission and 21 of them had received both chemotherapy and ATRA. However, there appeared to be no survival benefit from adding chemotherapy in maintenance.

The limitation of this study was the relatively small patient number. Another study had pointed out that FLT3-ITD mutation was also a poor prognostic factor[17], which was also lacking in our study.

In conclusion, our study showed that initial high WBC count and relapse were 2 major factors associated with poor prognosis and early mortality in patients undergoing conventional ATRA plus chemotherapy treatment. Early and deep molecular response after induction chemotherapy predicts reduced relapse and was a good prognostic factor.

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